Supplementary Discussion

ATP binding by UPF1 is required for the functional interaction with terminating ribosomes

Our observation that 3' RNA decay fragments detected in an ATP hydrolysis mutant of yeast UPF1 (i.e. DE572AA) do not also accumulate in cells expressing ATP binding-deficient UPF1 (K436E) was unexpected in the context of results that similar mutations in human UPF1 behave alike in studies evaluating their role in mRNP remodeling[1] and NMD target discrimination[2]. In an attempt to understand this discrepancy, three additional missense mutations were introduced at lysine position 436 – alanine, proline, or glutamine - all of which have been previously reported to inactivate the ATP binding activity of yeast UPF1[3]. Consistent with the work of Weng and colleagues in characterizing these mutants, we show that each is defective in targeting PTC-containing mRNA to NMD as demonstrated by the increased steady-state abundance of the endogenous NMD substrate, *CYH2* pre-mRNA[4] (Supplementary Fig. 1c, compare mutants to WT). Notably, consistent with our results with mutant UPF1 encoding glutamic acid at position 436 (i.e. K436E), we did not observe a 3' RNA decay intermediate in mutants where lysine was replaced with either proline or glutamine (K436P and K436Q, respectively; Supplementary Fig. 1d). In contrast, introduction of alanine did result in accumulation of an RNA intermediate similar in size to that formed in cells harboring ATPase-deficient UPF1 (compare DE and KA samples).

Our observation that four inactivating mutations in UPF1 that introduce different substitutions at lysine 436 each predicted to impair ATP binding did not behave similarly in our assay was unexpected. It is important to note, however, that not all of these UPF1 mutants have been characterized biochemically for their ATP binding activities, and that the initial analysis of these mutants was limited to demonstrating that they are defective in targeting substrates to NMD in yeast[3]. Critically, rigorous measurement of ATP binding activity has only been reported for one of the four mutants, K436Q[5], suggesting that the kinetics of ATP binding by the K436A mutant (or the others) has never been experimentally determined. Notably, the K436Q mutation was found to reduce the affinity of UPF1 for ATP 16-fold compared to wildtype, while ATP binding by the UPF1 DE572AA mutant was virtually identical to wildtype[5].

One simple interpretation of our data shown in Supplementary Fig. 1d is that the K436A mutant retains residual ATP binding activity *in vivo* that is sufficient to mediate the function of UPF1 on a prematurely terminating ribosome. We note that human UPF1 harboring the corresponding mutation (replacement of lysine 498 to alanine; K498A) retains ~5% ATP binding activity as measured by a semi-quantitative, *in vitro* steady-state binding assay[6]. We speculate that this residual ATP binding activity exhibited by the hUPF1 K498A mutant *in vitro* is sufficient to provide functional ATP binding activity *in vivo*, albeit at a reduced level. Consistent with this, the abundance of the 3' RNA decay fragments in cells expressing the UPF1 K436A mutant are 2-3 fold lower then levels found in the DE572AA mutant (Supplementary Fig. 1d).

Our findings are consistent with ATP binding and ATP hydrolysis activities of UPF1 having distinct functions in modulating translation termination at a PTC.

Additionally, our data reveal that while a number of missense mutations in UPF1 predicted to block ATP binding all function to inactivate the NMD pathway, they are not equivalent. Given the observation that the molecular phenotype of the UPF1 K436A mutant is distinct from variants with three other substitutions at this position calls into question the generality of conclusions drawn from experiments examining this particular mutant. It is important to highlight that a number of studies on the role of ATP binding and hydrolysis by human UPF1 in NMD have employed the K498A mutant[1,2]; based on our findings in yeast employing multiple mutations at the conserved residue, it will be of interest to assess additional mutations in hUPF1 to determine if they, too, exert different outcomes on recognition and/or degradation of NMD substrates, and establish whether ATP binding and ATP hydrolysis also have distinct functions in this pathway in mammals.

Depletion of RLI1 inhibits ribosome recycling and leads to accumulation of 3' RNA decay fragments similar to those observed in UPF1 ATPase mutants

Polyribosome analysis demonstrated that 3' RNA decay fragments accumulating in cells expressing ATPase-deficient UPF1 co-sediment with 80S ribosomes, indicating that they are bound by a single ribosome (Fig. 3b). Additionally, progressively larger RNA decay fragments were observed to sediment in increasingly dense fractions of the sucrose gradient, consistent with the piling up of a heightened number of ribosomes upstream of the nonsense codon that impose an ever more 5'-proximal block to XRN1 activity during co-translational decay of the mRNA[7,8] (Supplementary Fig. 4a). To lend support to our conclusion that the observed 3' RNA decay fragments result from a

ribosome stalled during translation termination which, in turn, present a block to 5' → 3' decay, we examined whether a defect in translation termination generated by an independent mechanism would also result in the accumulation of 3' RNA fragments.

As a means to demonstrate that inhibition of translation termination is sufficient to cause the accumulation of 3' RNA decay fragments, termination was blocked by depletion of RLI1, a conserved ABC-type ATPase required for stimulating peptide release *in vitro* and ribosome subunit dissociation during translation termination *in vivo*[9,10]. Similar to published methods[11] chromosomally-encoded *RLI1* was placed under control of a galactose-inducible promoter (P_{GAL1}-*3HA-RLI1*) and expression inhibited by growth of cells in media lacking galactose (see Methods). Eight to ten hours after inhibition of transcription, RLI1 protein abundance was <1% of steady-state levels and culture growth was dramatically impaired, consistent with an essential role for RLI1 in cell viability[11] (Supplementary Fig. 4b,c). Notably, depletion of RLI1 resulted in the accumulation of a 3' RNA fragment from PTC-containing *GFP* reporter mRNA comparable to the fragment generated in cells expressing ATPase-deficient UPF1 (Supplementary Fig. 4d). These data demonstrate that inhibition of translation termination is sufficient to lead to accumulation of 3' RNA decay fragments, strongly supporting our interpretation that the RNA fragments detected in UPF1 mutants arise as a consequence of a stalled ribosome caused by failure of UPF1 to hydrolyze ATP.

We note that the RNA fragments generated upon depletion of RLI1 are heterogeneous and reduced in size relative to the 3' RNA decay fragment in UPF1 mutant cells (Supplementary Fig. 4d). This difference is likely due to transit of ribosomes past termination codons to various positions within mRNA 3' UTRs, as

previously observed by ribosome profiling in cells depleted for RLI1[11]. Such transited ribosomes would permit XRN1 to degrade the transcript beyond the nonsense codon leading to the production of the smaller and heterogeneous 3' RNA decay fragments, as we observe.

Direct binding of UPF1 by UPF2 is required for UPF1 function in translation termination

We observe a requirement for UPF2 (and UPF3) in the accumulation of 3' RNA decay fragments in ATPase-deficient UPF1 mutants (Fig. 4B). To assess whether the function of UPF2 is mediated through direct binding to UPF1, two modifications were introduced into UPF1 previously shown to be sufficient to disrupt the physical interaction between these two proteins[12,13]. Notably, either deletion of the entire CH-domain of UPF1 (amino acids 62-212) or a point mutation within this region (C62Y) resulted in the loss of the 3' RNA decay fragments when introduced into ATPase-deficient UPF1 mutants (Supplementary Fig. 5). These results show that the requirement for UPF2 in UPF1 function is mediated through a direct interaction with UPF1 via its N-terminal CH domain.

Biochemical and structural studies reveal that UPF1 undergoes a conformational rearrangement upon UPF2 binding that alleviates allosteric inhibition caused by an intramolecular interaction between the UPF1 CH and helicase domains and results in enhanced ATPase and helicase activities *in vitro* [14]. The intramolecular interaction within UPF1 is mediated, in part, by a conserved phenylalanine residue (i.e. F131) located at the interface; importantly, mutation of this residue to glutamic acid has been

shown to destabilize contact between the two domains and bypass the requirement for UPF2 binding in activating UPF1[14]. We reasoned, therefore, that introducing the F131E mutation into ATPase-deficient UPF1 may be sufficient to overcome the requirement for UPF2 in the function of mutant UPF1 on termination. Notably, in cells expressing UPF2, introduction of the F131E mutation did not alter the ability of ATPase-deficient UPF1 to promote 3' RNA fragment accumulation in the presence of UPF2 (Fig 4c, lane 3). However, the mutation also did not bypass of the requirement for UPF2 in our assay (Fig. 4c, lane 6). While it is possible that the F131E mutation does not impose a consequence on UPF1 function *in vivo* as is observed *in vitro*, we deem this unlikely given that mutation of the conserved residue in human UPF1 reduces RNA binding activity of the protein[2]. These data suggest that UPF2 likely plays a novel role in NMD and in regulating UPF1 function that is independent of its ability to induce conformational changes to UPF1 that stimulate its helicase activity.

Supplementary References

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Supplementary Tables

Supplementary Table 1. Yeast strains used in this study

NAME	GENOTYPE	NOTES	REFERENCE
yKB146	MATa*, ura3, leu2, his3, met15, upf1::KanMX*	*upf1*Δ	EUROSCARF
yKB154	MATa*, ura3, leu2, his3, met15*	Wild-type	EUROSCARF
yKB227 (yRP2076)	MATa*, ura3, leu2, his3, met15, lys2, upf1::KanMX, xrn1::KanMX*	*upf1*Δ/*xrn1*Δ	Doma and Parker, 2006
yKB478	MATa*, ura3, leu2, his3, met15, upf1::MET15, upf3::KanMX*	*upf1*Δ/*upf3*Δ	This study
yKB479	MATa*, ura3, leu2, his3, met15, upf1::KanMX, upf2::MET15*	*upf1*Δ/*upf2*Δ	This study
yKB494	MATa, *ura3, leu2, his3, met15, UPF1:HA-HIS3*	HA tagged *UPF1*	This study
yKB498	MATa*, ura3, leu2, his3, met15, upf1::MET15 upf2::HIS3, upf3::KanMX*	*upf1*Δ/*upf2*Δ/*upf3*Δ	This study
yKB704	MATa*, ura3, leu2, his3, met15, HIS3-*P_{GAL1}*-3HA-RLI1*	HA tagged *RLI1* under control of *GAL1* promoter	This study

Supplementary Table 2. Plasmids used in this study

NAME	DESCRIPTION	NOTES	REFERENCE
pFA6a-His3MX6-PGAL1-3HA	Cassette used for tagging and placing *RLI1* under control of *GAL* promoter		Longtine et al., 1998
pJC408	*PGK1* with MS2 sites in 3' UTR, under control of *GAL1* promoter*; URA3, CEN*	Used for construction of pKB581, pKB589, pKB593.	Sweet et al., 2012
pJC424	*SL-PGK1* with MS2 sites, under control of *GAL1* promoter; *URA3, CEN*	Used for construction of pKB590	Sweet et al., 2012
pKB102 (YEplac181)	*LEU2, 2μ*	Used for construction of pKB598	Geitz and Sugino, 1988
pKB105 (YCplac111)	*LEU2, CEN*	Used for construction of pKB556	Geitz and Sugino, 1988
pKB290	*GFP* under control of *GAL1* promoter*; URA3, CEN*	Used for construction of pKB303, pKB311, pKB312	Baker and Parker, 2006
pKB303	*GFP-PTC 67* under control of *GAL1* promoter; *URA3, CEN*		Baker and Parker, 2006
pKB311	*GFP-PTC 135* under control of *GAL1* promoter; *URA3, CEN*		This study
pKB312	*GFP-PTC 200* under control of *GAL1* promoter; *URA3, CEN*		This study
pKB509	*GFP* under control of *TDH3* promoter; *URA3, CEN*	Used for construction of pKB510	This study
pKB510	*GFP-PTC 135* under control of *TDH3* promoter; *URA3, CEN*		This study
pKB556	*UPF1-HA; LEU2, CEN*		This study
pKB576	*UPF1-HA-DE572AA*; *LEU2, CEN*		This study
pKB578	*UPF1-HA-K436E*; *LEU2, CEN*		This study
pKB579	*UPF1-HA-RR793AA; LEU2, CEN*		This study
pKB581	*PGK1-PTC 142* with MS2 sites in 3' UTR, under control of *GAL1* promoter; *URA3, CEN*		This study
pKB589	*PGK1-PTC 344* with MS2 sites in 3' UTR, under control of *GAL1* promoter; *URA3, CEN*		This study
pKB590	*SL-PGK1-PTC142* with MS2 sites in 3' UTR, under control of *GAL1* promoter; *URA3, CEN*		This study
pKB593	*PGK1-PTC 225* with MS2 sites in 3' UTR, under control of *GAL1* promoter; *URA3, CEN*		This study
pKB598	*UPF1; LEU2, 2μ*		This study
pKB607	*UPF1-DE572AA; LEU2, 2μ*		This study
pKB610	*UPF1-DE572AA/RR793AA; LEU2, 2μ*		This study
pKB621	*UPF1-C62Y/DE572AA; LEU2, 2μ*		This study
pKB638	*UPF1-ΔCH; LEU2, 2μ*		This study
pKB640	*UPF1-F131E; LEU2, 2μ*		This study
pKB641	*UPF1-F131E/DE572AA; LEU2, 2μ*		This study
pKB642	*UPF1-C62Y; LEU2, 2μ*		This study

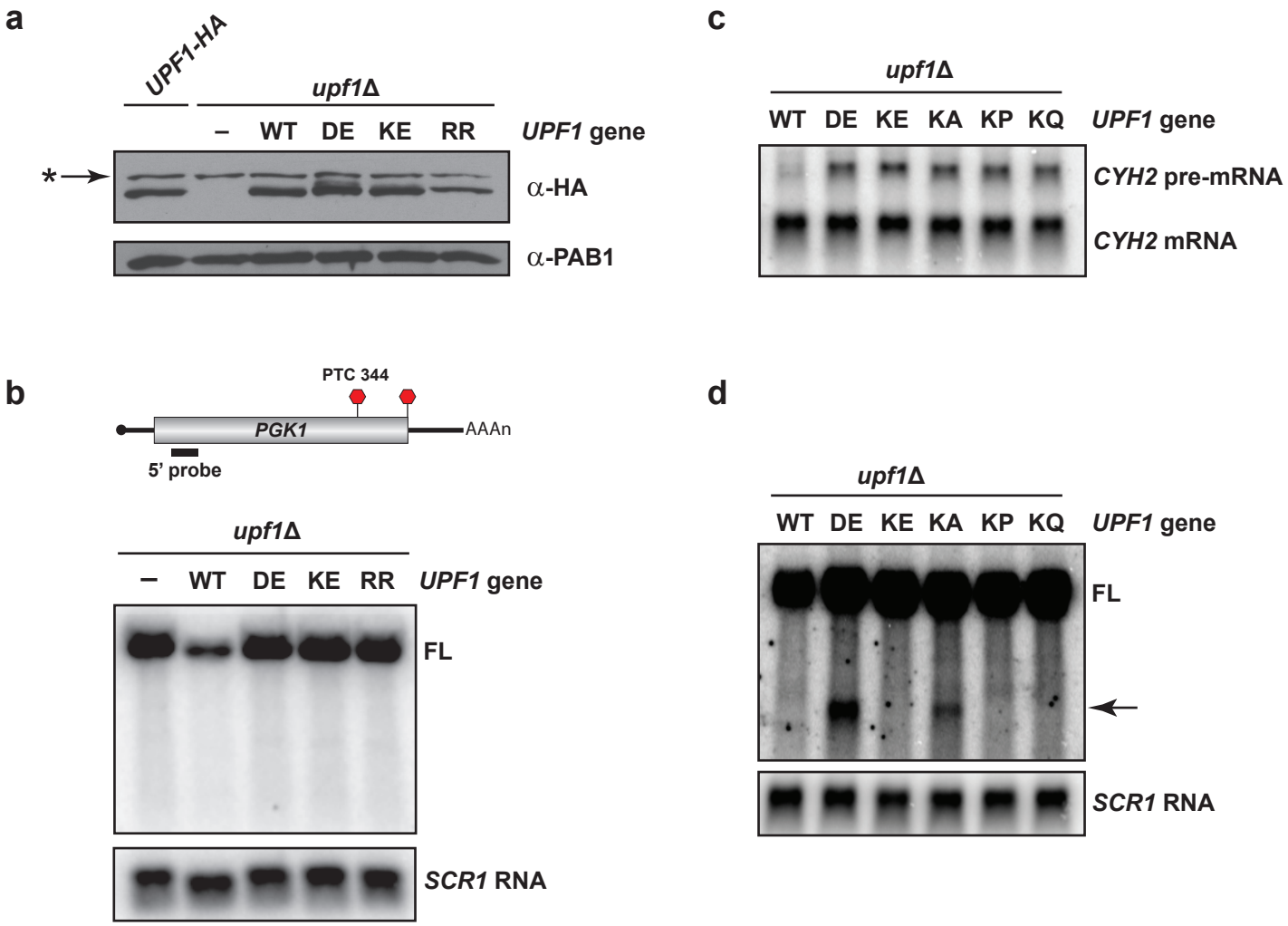
pKB645	*UPF1-ΔCH-DE572AA; LEU2, 2μ*		This study
pKB690	*UPF1-HA-K436P*; *LEU2, CEN*		This study
pKB691	*UPF1-HA-K436Q*; *LEU2, CEN*		This study
pKB692	*UPF1-HA-K436A*; *LEU2, CEN*		This study

Supplementary Table 3. Oligonucleotides used in this study

NAME	SEQUENCE	NOTES	REFERENCE
oJC306	GTCTAGCCGCGAGGAAGG	*SCR1* oligo probe	Smith et al., 2014
oJC1006	AGACATGGGTGATCCTCATG	*MS2* oligo probe; used as 3' UTR probe for *PGK1* reporters.	Sweet et al., 2012
oKB147	CCATACCTCTACCACCGGGGTGCTTTC TGTGCTTACCG	*CYH2* oligo probe	Meaux et al., 2008
oKB245	GGCCAACACTTGTCACTACTTTCACTTA AGGTGTTCAGTGCTTTTCAAGATACCC GGATC	Forward mutagenesis primer for inserting PTC at codon 67 of *GFP* mRNA	This study
oKB246	GGCCAACACTTGTCACTACTTTCACTTA AGGTGTTCAGTGCTTTTCAAGATACCC GGATC	Reverse mutagenesis primer for inserting PTC at codon 67 of *GFP* mRNA	This study
oKB227	GAGTTAAAAGGTATTGATTTTAAAGAAG ATTAAAACATTCTTGGGCACAAATTGGA ATAC	Forward mutagenesis primer for inserting PTC at codon 135 of *GFP* mRNA	This study
oKB228	GTATTCCAATTTGTGCCCAAGAATGTTT TAATCTTCTTTAAAATCAATACCTTTTAA CTC	Reverse mutagenesis primer for inserting PTC at codon 135 of *GFP* mRNA	This study
oKB229	GGCCCTGTCCTTTTACCAGACAACCAT TAACTGTCCACACAATCTGCCCTTTCGA AAG	Forward mutagenesis primer for inserting PTC at codon 200 of *GFP* mRNA	This study
oKB230	CTTTCGAAAGGGCAGATTGTGTGGACA GTTAATGGTTGTCTGGTAAAAGGACAG GGCC	Reverse mutagenesis primer for inserting PTC at codon 200 of *GFP* mRNA	This study
oKB397	CCACCAGGTGTTTTCGAATTCTAAAAGT TCGCTGCTGGTACTAAGG	Forward mutagenesis primer for inserting PTC at codon 344 of *PGK1* mRNA	This study
oKB398	CCTTAGTACCAGCAGCGAACTTTTAGA ATTCGAAAACACCTGGTGG	Reverse mutagenesis primer for inserting PTC at codon 344 of *PGK1* mRNA	This study
oKB454	GAATTCGTGACGACGCGTAAGCTTGTC GACTTAGCAGCCAGATCCTTTGTATAG	*GFP* oligo probe	This study
oKB823	ACTAAATTTAGGACTGTGTTAATTGCTG CAAGTACTCAAGCTTCTGAGCCGG	Forward mutagenesis primer for UPF1-DE572AA mutation	This study
oKB824	CCGGCTCAGAAGCTTGAGTACTTGCAG CAATTAACACAGTCCTAAATTTAGT	Reverse mutagenesis primer for UPF1-DE572AA mutation	This study

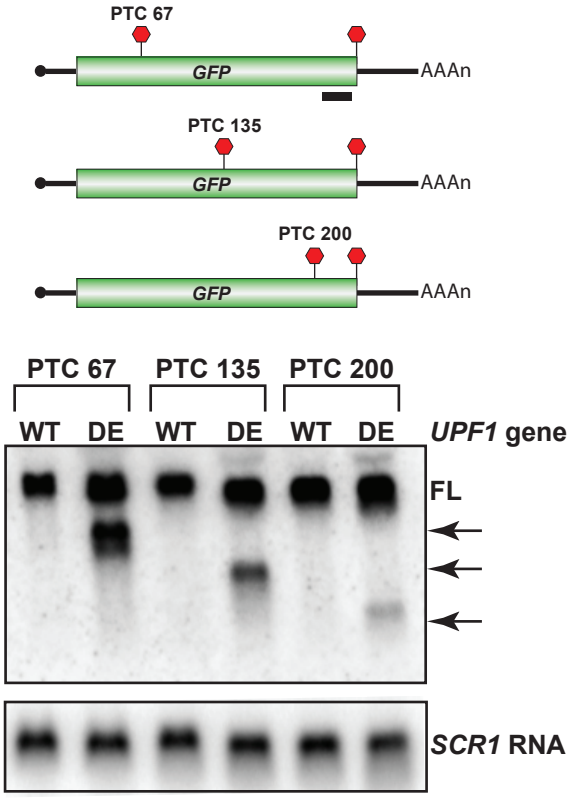
oKB825	AATTCAAGGCCCACCAGGCACTGGTGAAACAGTTACTTCAGCAACGATTG	Forward mutagenesis primer for UPF1-K436E	This study
oKB826	CAATCGTTGCTGAAGTAACTGTTTCACCAGTGCCTGGTGGGCCTTGAATT	Reverse mutagenesis primer for UPF1-K436E mutation	This study
oKB827	AATGGTTTCTTACGTGATCCTGCTGCTCTAAACGTGGGTCTAACCCGTGC	Forward mutagenesis primer for UPF1-RR793AA mutation	This study
oKB828	GCACGGGTTAGACCCACGTTTAGAGCAGCAGGATCACGTAAGAAACCAAT	Reverse mutagenesis primer for UPF1-RR793AA mutation	This study
oKB829	CCTTCAGCTTCAGACAATTCATACGCGTATTGTGGTATAGATTC	Forward mutagenesis primer for UPF1-C62Y mutation	This study
oKB830	GAATCTATACCACAATACGCGTATGAATTGTCTGAAGCTGAAGG	Reverse mutagenesis primer for UPF1-C62Y mutation	This study
oKB849	CTACTGCAGTCACTAGCGAGTTCATTCTCCA	Forward PCR primer for amplification of *UPF1* ± 500 bp for making pKB598	This study
oKB850	CTAGGATCCGGCATAGTTCACACTTTTATCTCC	Reverse PCR primer for amplification of *UPF1* ± 500 bp for making pKB598	This study
oKB891	GTCAAAAGGTCAAGGCTTCCTAGGAAGATGTTCAAAAGTTC	Forward mutagenesis primer for inserting PTC at codon 142 of *PGK1* mRNA	This study
oKB892	GAACTTTTGAACATCTTCCTAGGAAGCCTTGACCTTTTGAC	Reverse mutagenesis primer for inserting PTC at codon 142 of *PGK1* mRNA	This study
oKB908	GATTCAATTGATTGACAACTAGTTGGACAAGGTCGACTC	Forward mutagenesis primer for inserting PTC at codon 225 of *PGK1* mRNA	This study
oKB909	GAGTCGACCTTGTCCAACTAGTTGTCAATCAATTGAATC	Reverse mutagenesis primer for inserting PTC at codon 225 of *PGK1* mRNA	This study
oKB1031	TCAGCTTCAGACAATTCAACTAGTTGTGCGTATTGTGGTATA	Forward mutagenesis primer for insertion of SpeI site at 5’ end of CH domain	This study
oKB1032	TATACCACAATACGCACAACTAGTTGAATTGTCTGAAGCTGA	Reverse mutagenesis primer for insertion of SpeI site at 5’ end of CH domain	This study
oKB1097	GATCCAATAAAGACGCTACAACTAGTATTAATGATATTGACGCCCCAGA	Forward mutagenesis primer for insertion of SpeI site at 3’ end of CH domain	This study
oKB1098	TCTGGGGCGTCAATATCATTAATACTAGTTGTAGCGTCTTTATTGGATC	Reverse mutagenesis primer for insertion of SpeI site at 3’ end of CH domain	This study
oKB1123	TTCAGCGCGAATTCCACCTAGCCTTCTGCAAAAGTTCTTAAGAAAACACGACAGACCATAGAATTCGAGCTCGTTTAAAC	Forward PCR primer used for amplification of 3HA-GAL1 from pFA6a-His3MX6-PGAL1-3HA, with gene specific sequences for *RLI1*	This study
oKB1124	CTTTTTTGGTTTACATTTATCAGCGCTAACGATAGCGATACGACTGTTTTTATCAC	Reverse PCR primer used for amplification of 3HA-	This study

	TCATGCACTGAGCAGCGTAATCTG	GAL1 from pFA6a-His3MX6-PGAL1-3HA, with gene specific sequences for *RLI1*	
oKB1144	CTGTGGACGTAAGAACGTGGAATTATTGGGATTTGTTTCCG	Forward mutagenesis primer for UPF1-F131E mutation	This study
oKB1145	CGGAAACAAATCCCAATAATTCCACGTTCTTACGTCCACAG	Reverse mutagenesis primer for UPF1-F131E mutation	This study
oKB1156	CTTAGCAACTGGAGCCAAAGA	*PGK1* oligo probe; used as 5' ORF probe for *PGK1* reporters.	This study
oKB1324	CCACCAGGCACTGGTGCAACAGTTACTTCAGC	Forward mutagenesis primer for UPF1-K436A mutation	This study
oKB1325	GCTGAAGTAACTGTTGCACCAGTGCCTGGTGG	Reverse mutagenesis primer for UPF1-K436A mutation	This study
oKB1326	CCACCAGGCACTGGTCCAACAGTTACTTCAGC	Forward mutagenesis primer for UPF1-K436P mutation	This study
oKB1327	GCTGAAGTAACTGTTGGACCAGTGCCTGGTGG	Reverse mutagenesis primer for UPF1-K436P mutation	This study
oKB1328	CCACCAGGCACTGGTCAAACAGTTACTTCAGC	Forward mutagenesis primer for UPF1-K436Q mutation	This study
oKB1329	GCTGAAGTAACTGTTTGACCAGTGCCTGGTGG	Reverse mutagenesis primer for UPF1-K436Q mutation	This study



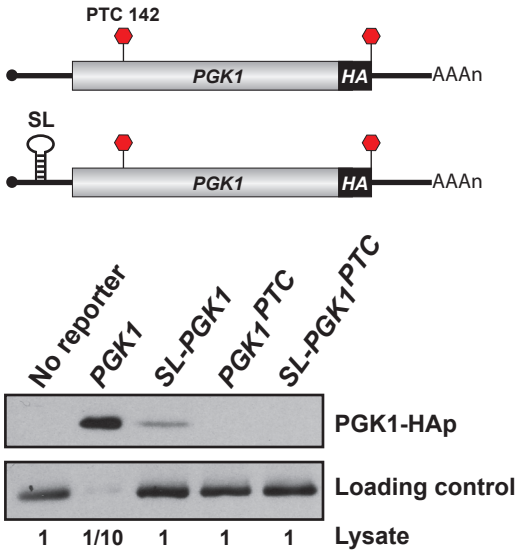
Supplementary Figure 1. 3' RNA decay fragments observed in ATPase-deficient UPF1 mutants are not generated in UPF1 ATP binding mutants with the exception of UPF1 K436A

(**a**) Western blot analysis of epitope-tagged UPF1 expressed either from the chromosome (*UPF1-HA*) or from single-copy plasmids in *upf1Δ* cells. Protein levels were normalized to poly(A) binding protein (PAB1). A non-specific band cross-reacting with the α-HA antibody is indicated (asterisk). (**b**) Northern blot analysis of PTC-containing *PGK1* reporter mRNA in *upf1*Δ cells (-) complemented with wild-type or mutant *UPF1* detected using a probe complementary to a 5' region of the mRNA. (**c**) Northern analysis of *CYH2* RNA from *upf1Δ* cells expressing wild-type (WT), ATPase-deficient (DE), or putative ATP binding-deficient (KE, KA, KP, KQ) UPF1. NMD-sensitive *CYH2* pre-mRNA and NMD insensitive mRNA are indicated. (**d**) As in **c** but probing for *PGK1-PTC344* reporter mRNA. Full-length reporter mRNA (FL) and fragments (arrow) are indicated. RNA levels were normalized to NMD-insensitive *SCR1* mRNA.



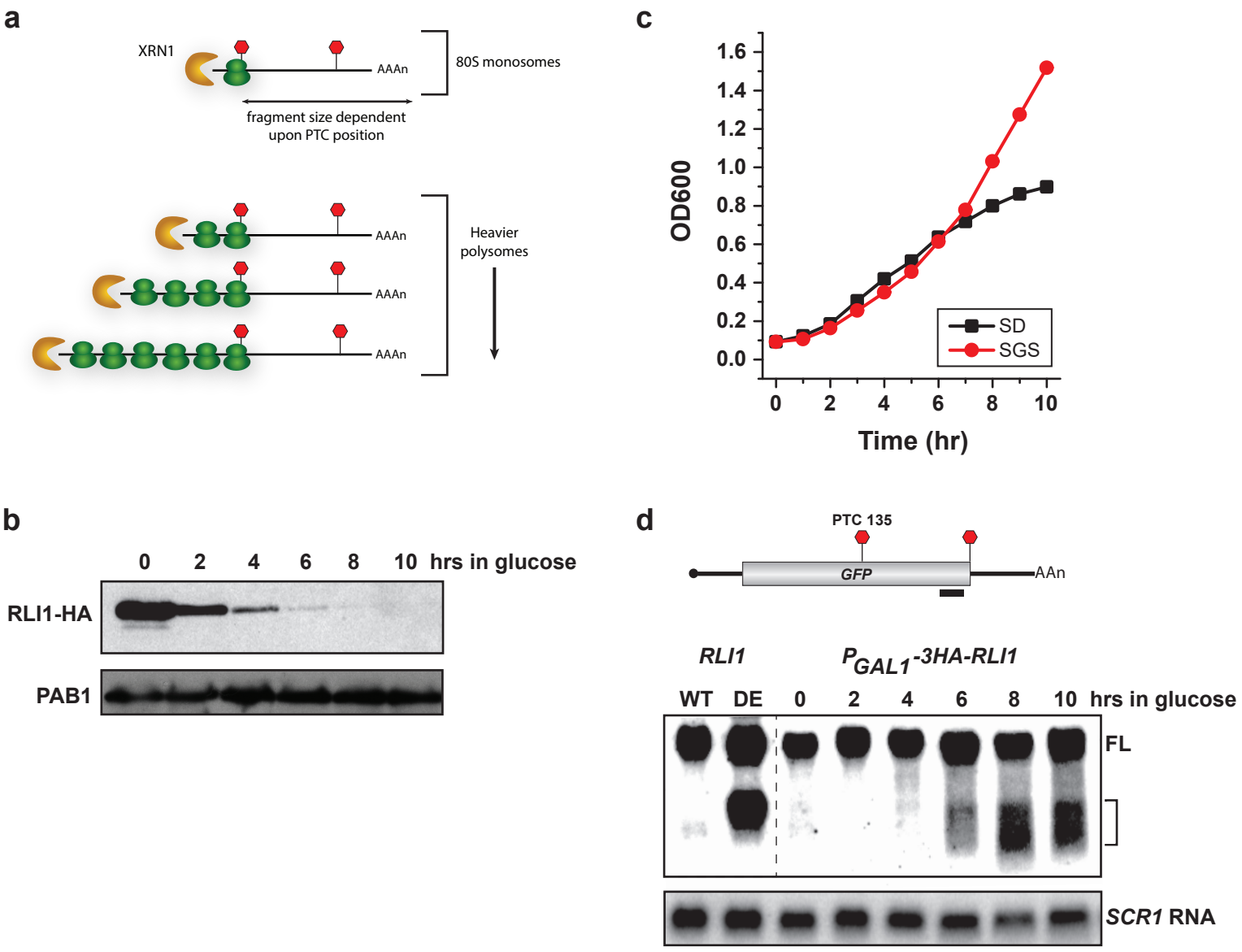
Supplementary Figure 2. 3' RNA fragments accumulate in a PTC position-dependent but substrate-independent manner

Northern analysis of *GFP* reporter mRNAs harboring PTCs at one of three indicated positions in *upf1*Δ cells expressing wild-type (WT) or ATPase-deficient (DE) UPF1. Full-length reporter mRNA (FL) and fragments (arrow) are indicated. RNA levels were normalized to NMD-insensitive *SCR1* mRNA.



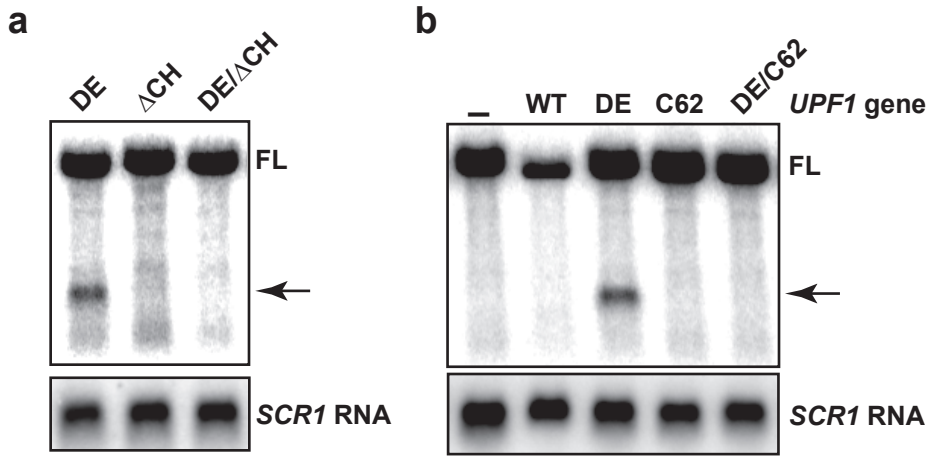
Supplementary Figure 3. Translation of *PGK1* reporter mRNA is strongly inhibited by insertion of a stable stemloop structure in the 5' UTR

Western blot analysis of epitope-tagged PGK1 expressed from normal or PTC-containing reporter genes either lacking (i.e. *PGK1* or *PGK1*^{PTC}) or harboring a stable stemloop structure (SL) in the 5' UTR (i.e. *SL-PGK1* or *SL-PGK1*^{PTC}). Based on sample loading (normalized to a loading control), translation of stemloop-containing *PGK1* mRNA was inhibited >100-fold; efficient termination at the PTC resulted in no detectable full-length protein.



Supplementary Figure 4. Inhibition of translation termination induces formation of a 3' RNA decay fragment by co-translational mRNA decay

(**a**) Co-translational decay of PTC-containing mRNA inhibited for translation termination by ATPase-deficient UPF1 results in a build-up of ribosomes that present a greater 5' proximal block to XRN1 activity and the formation of increasingly larger 3' RNA decay fragments that co-sediment with heavier polyribosomes. (**b**) Western blot analysis of epitope-tagged RLI1 after transcriptional inhibition [in hours]. Protein levels were normalized to PAB1. (**c**) Growth of P_{GAL1}-*3HA-RLI1* cells under transcriptionally active (SGS, red line) or inactive (SD, black line) conditions. (**d**) Northern blot analysis of *GFP-PTC135* reporter mRNA in *upf1Δ* cells expressing wild-type (WT) or ATPase-deficient (DE) UPF1 (lanes 1 and 2), or P_{GAL1}-*3HA-RLI1* cells (expressing endogenous *UPF1*) after transcriptional inhibition for the indicated amount of time (lanes 3 – 8). RNA levels were normalized to NMD-insensitive *SCR1* RNA.



Supplementary Figure 5. Interaction with UPF2 is required for UPF1 function in premature translation termination

(**a**) Northern blot analysis of *PGK1-PTC344* reporter mRNA in *upf1Δ* cells expressing ATPase-deficient UPF1 (DE), UPF1 lacking the N-terminal cysteine/histidine rich domain (ΔCH), or UPF1 with both alterations (ΔCH/DE). (**b**) Northern blot analysis of *PGK1-PTC344* reporter mRNA in *upf1Δ* cells expressing ATPase-deficient UPF1 (DE), mutant UPF1 defective in binding to UPF2 (C62), or UPF1 with both mutations (C62/DE). Full-length (FL) reporter RNA and fragments (arrow) indicated. RNA levels were normalized to NMD-insensitive *SCR1* mRNA.

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Uncropped Images Related to Figure 1

Figure 1b; PGK1

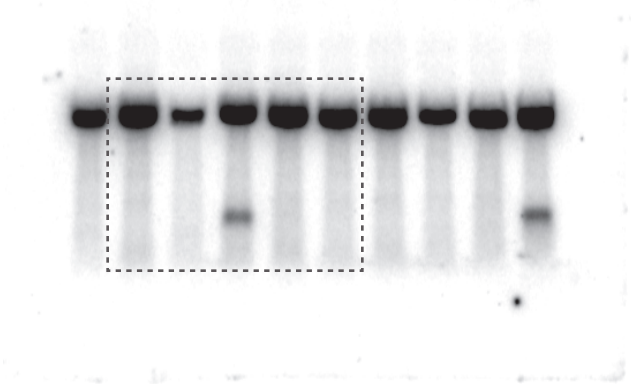


Figure 1b; SCR1

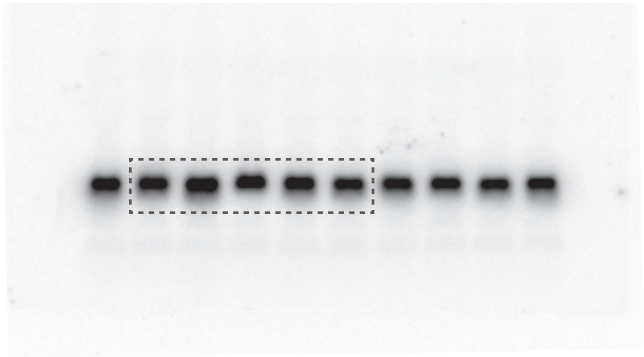


Figure 1c; PGK1

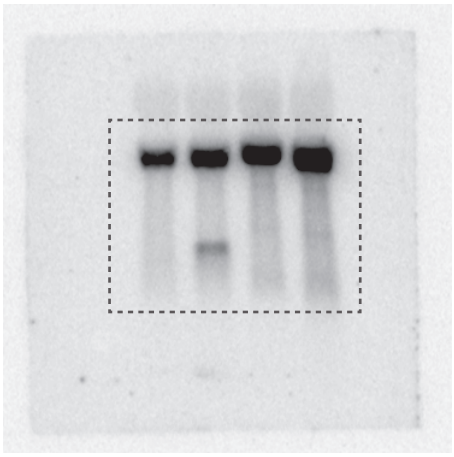


Figure 1c; SCR1

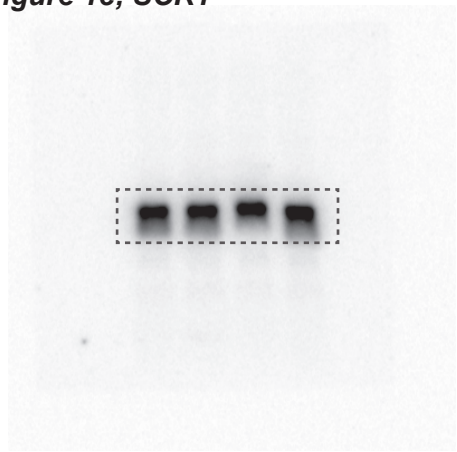


Figure 1d; PGK1

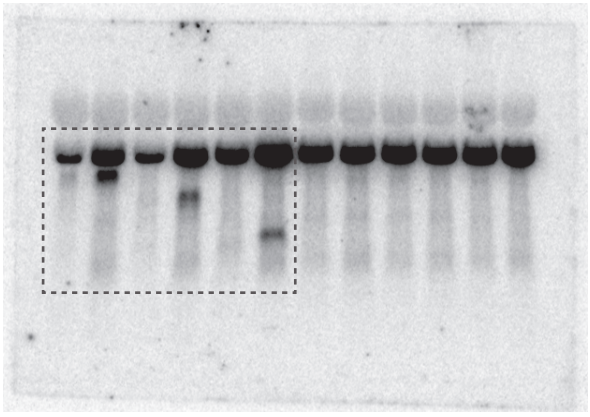
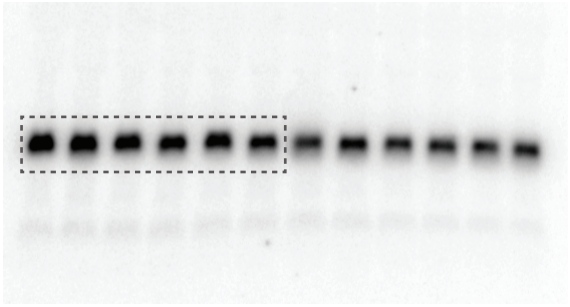


Figure 1d; SCR1



Uncropped Images Related to Figure 2

Figure 2a; PGK1

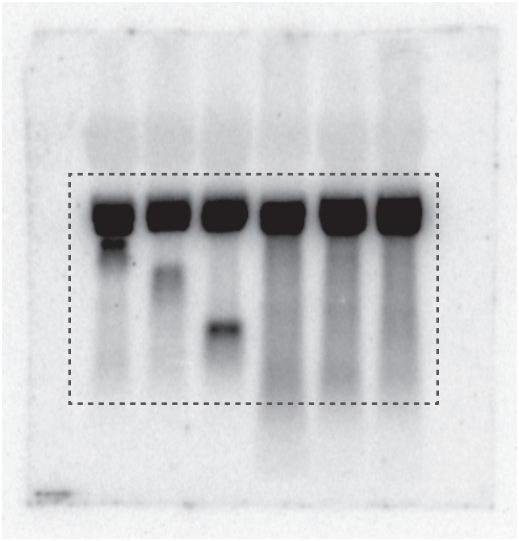


Figure 2b; PGK1

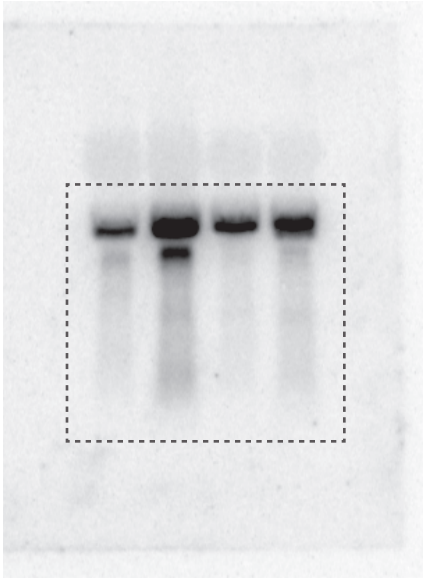


Figure 2a; SCR1

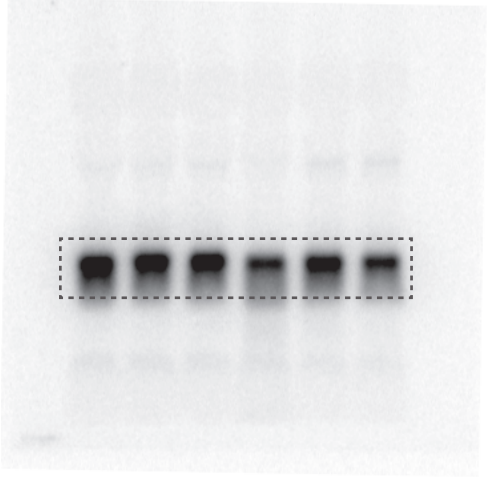
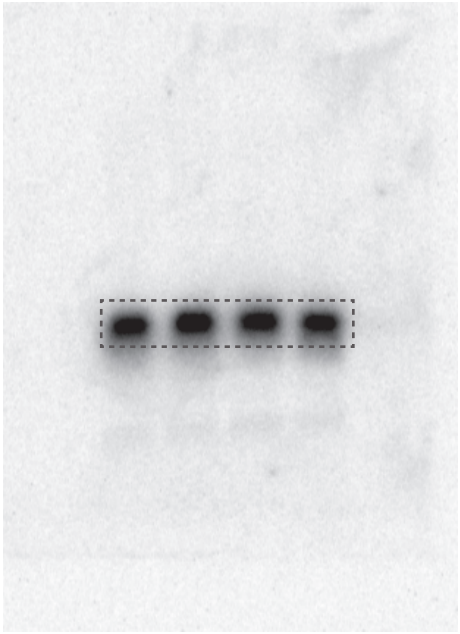
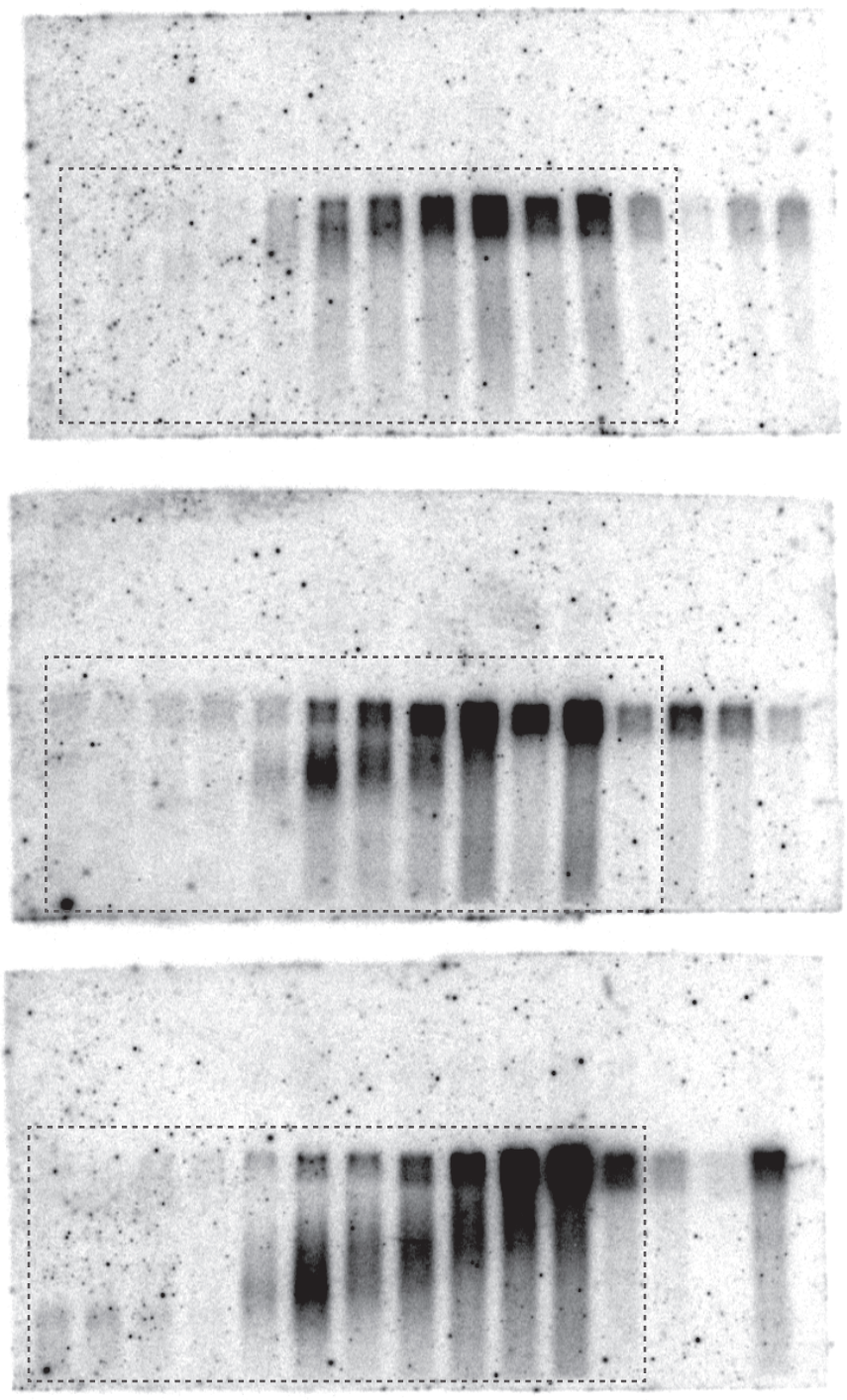


Figure 2b; SCR1



Uncropped Images Related to Figure 3

Figure 3b; PGK1



Uncropped Images Related to Figure 4

Figure 4a; PGK1

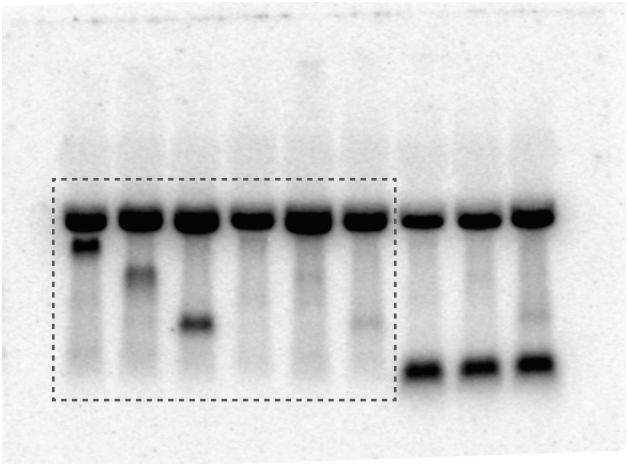


Figure 4a; SCR1

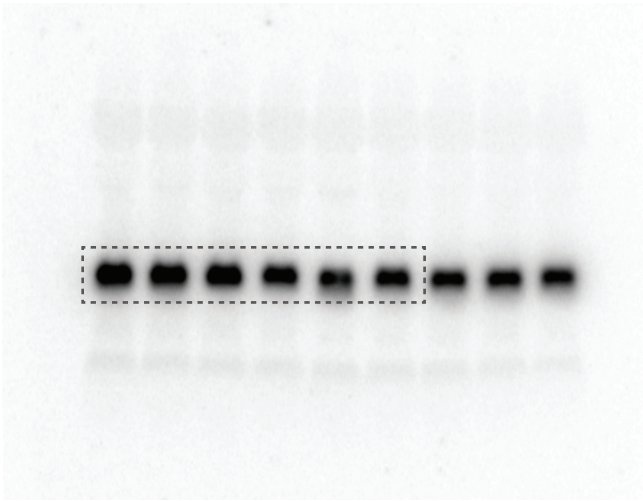
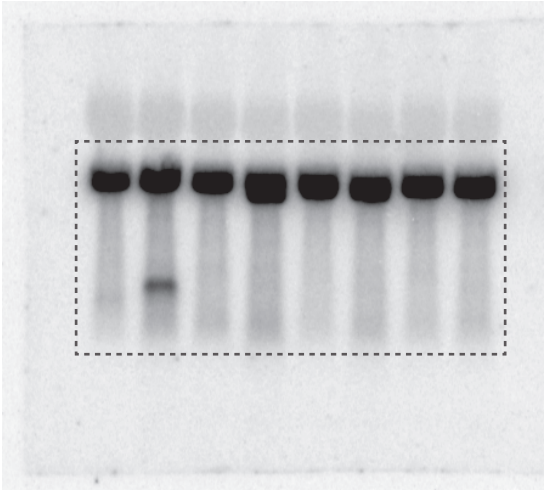


Figure 4b; PGK1



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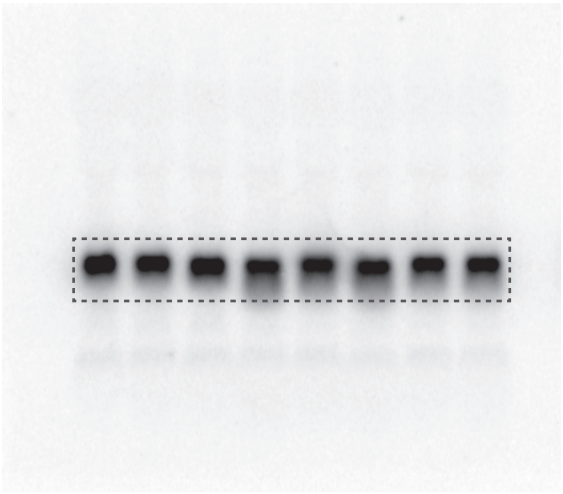

Figure 4c; PGK1

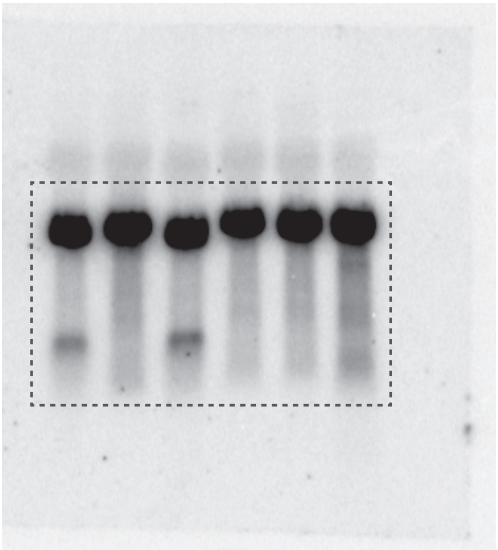

Figure 4c; SCR1